Mini Review
Sciences of Phytochemistry

A Mini Review on the Analytical Method and Its Validation for *Psidium guajava*

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Abstract: The well-known tropical tree *Psidium guajava*, also known as the guava, is widely farmed for its fruit. This article consists of literature about various analytical techniques like TLC, UV spectrophotometry, HPLC, and HPTLC that could be used to analyze the Guava plant. The HPLC analysis of Quercetin isolated from the leaves of the guava plant uses a 4 x 125 mm Hypersil ODS column with a mobile phase of 0.5 percent ortho-phosphoric acid in water and methanol at a flow rate of 1 ml/min. The column wash times are 20 min and 18 min, respectively. 250C is the temperature in the column. Silica gel 60F 254 pre-coated plates (10 x 10 cm) were used with toluene: acetone: formic acid (38:10:5) as the solvent system for HPTLC analysis of quercetin obtained from the tender leaves of guava. The λ max was observed in the U.V. spectrum of the isolated compound (quercetin) on three standard wavelengths: 212 nm, 256 nm, and 372 nm. An effort can be made to produce a new analytical method that will be cost-effective and time-saving.

Introduction

Natural products from plants, animals, and minerals have been the basis of the treatment of human disease. The World Health Organization recommends and encourages the use of traditional herbs/remedies because a huge amount of raw material is easily available (1). Psidium guajava has a long history of being used medicinally. Common names for the plant include "Guava" in English, "Guayabo" in Spanish, "Goyave" and "Goyavier" in French, "Guyabaorgoeajab" in Dutch, "Goiaba" and "Goaibeira" in Portuguese, and "Jambubatu" in Malaya. *P. guajava* is a 6- to 25-foot-tall evergreen shrub or tree with leaves, flowers, fruit, seeds, bark, and other parts (2).

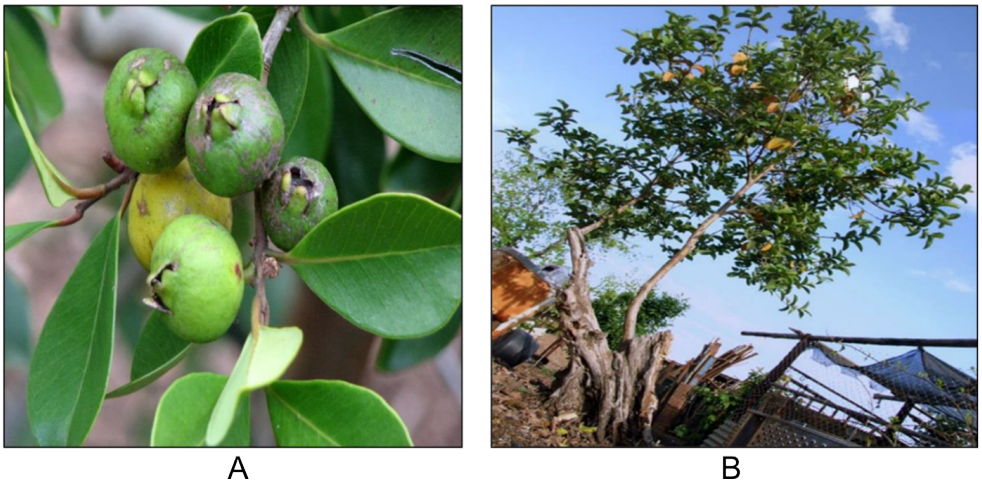

Figure 1. Images showing the *P. guajava* (L) where (A) Guava fruit (B) Guava tree i.e. Guava plant.

P. Guajava is a small tropical tree or shrub belonging to the family Myrtaceae (3). The fruit of P. guajava contains vitamins A and C, and minerals like iron, calcium, and phosphorus. The skin of the fruit contains ascorbic acid in very high amounts while the main fruit contains saponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin, quercetin, and other flavonoids (4).

The images of guava fruit, guava leaves, and guava tree is given in **Figure 1**. Guava leaves are abundant in flavonoids, especially quercetin. *P. guajava* leaves have recently yielded five ingredients, including a new pentacyclic triterpenoid called guajanoic acid and four previously identified substances called beta-sitosterol, uvaol, oleanolic acid, and ursolic acid. There are significant levels of tannins (11-27 %) in the guava tree's bark. Leucocyanidin, luectic acid, ellagic acid, and amritoside have been identified from the stem bark (5). The alcoholic root bark of *P. guajava* contains flavonoids, tannins, alkaloids, and saponins (6). Guava fruit was also reported to contain carotenoids (7). A total of 87.06 % of guava seed oil was found to be primarily composed of unsaturated fatty acids, namely oleic acid and linoleic acid. In the guava seed oil, monounsaturated fatty acids makeup about 10% of the total unsaturated fatty acids, and polyunsaturated fatty acids made up 77% present in the guava seed oil (8, 9).

P. guajava exhibits a wide range of pharmacological activities. The antioxidant activity of guava prevents lipid peroxidation (10). The water extract from the plant decreases the frequency of cough (11). The water extract of the plant shows anti-diabetic activity which possesses a hypoglycaemic effect (12). The aqueous and methanolic extracts of P. guajava bark were reported to show antibacterial properties (13). The aqueous leaf extracts from *P. guajava* exhibit good hepatoprotective action (14). Rats and mice were significantly protected against castor oil-induced diarrhoea by *P. guajava* leaf aqueous extract (15). The active flavonoid guaijaverin has shown excellent potential as an antiplaque agent (16). Direct-acting mutagens' mutagenicity was successfully inactivated by the *P. guajava* aqueous extract (17). P. guajava leaf extract reduces myocardial inotropism in Guinea pigs (18). The aglycone, quercetin showed spasmolytic activity (19). An aqueous extract of the budding leaves of guava showed anti-cancer activity (20). *P. guajava* leaf aqueous extract has analgesic and anti-inflammatory effects (21). Acne is treated with leaf extracts from *P. guajava* (22). The leaves of *P. guajava* showed antipyretic activity when extracted with methanol (23). *P. guajava* leaves aqueous extract showed antiplasmodial activity (24).

Analytical Method Development and Validation

Analytical Characterization of Quercetin

Thin Layer Chromatography for Quercetin

General Steps Involved in Performing TLC

Preparation of TLC plate: A thin layer was created on the TLC glass plates by arranging the adsorbent media (silica Gel-G) in a slurry of distilled water.

Activation of TLC plate: The TLC plate was activated by heating it in an oven at 105 °C for 30 minutes.

Sample application: After a capillary tube was dipped into the solution to be examined, the sample spot was air dried and placed on the sparse layer plate, approximately 2 cm from the base.

Chamber saturation: The glass TLC chamber needed to be saturated by ensuring complete absorption of the mobile phase. The mobile phase was added to the compartment and the lid was then put on, allowing it to saturate for about 30 minutes.

Chromatogram development: After saturating the chamber and placing the samples on the plate, the plate was kept in the chamber. It was important to ensure that the solvent level in the bottom of the chamber was lower than the spot applied on the plate, as the spotted material would dissolve in the pool of solvent rather than undergoing chromatography if the solvent level was higher. The solvent was allowed to run on the silica plate for about 10-15 cm.

Visualization: The plates were removed from the chamber and inspected under a UV cabinet. A suitable visualizing agent, such as Vanillin-sulphuric acid or Methanolic ferric chloride solution, was applied. Afterward, the Rf value was computed using the given formula.

Rf = Distance Travelled by Solute from Origin Line Distance Travelled by Solvent from Origin Line (25).

Solvent System of Thin Layer Chromatography

Table 1 refers to the solvent system of a TLC method to identify quercetin and uses the following solvents in its mobile phase: ethyl acetate, formic acid, glacial acetic acid (GAA), and water.

Table 1. Development system for TLC of Flavonoid (Quercetin) (25, 26).

Stationary phase	Silica gel-G
Mobile phase	Ethyl acetate: Formic acid: GAA: Water (100:11:11:26 v/v/v/v)
Chamber saturation	30 min
Visualization	Solution of Anisaldehyde – Sulphuric acid

UV of Quercetin

A total of 0.1 g of quercetin (1000 µg/ml) was dissolved in 100 ml of ethanol. After diluting the 0.1ml of stock solution with 100ml distilled water (10 µg/ml), the l max value was seen in **Table 2**.

Table 2. ƛ max of the Quercetin and isolated quercetin under UV (25, 27).

Standard	Isolated
Absorbance nm	Absorbance nm
212	205
256	256
372	368

HPLC

The HPLC analysis of quercetin was performed with a 4 x 125 mm Hypersil ODS column. The mobile phase consists of 0.5 percent ortho-phosphoric acid in a mixture of water and methanol, with a 1 ml/min flow rate. The column wash times are 20 min and 18 min, respectively. The column is maintained at a temperature of 25°C. A sample injection volume of 10 µL was used. During the HPLC analysis, both the peaks of the standard quercetin and the isolated compound from P. guajava Linn exhibited similar characteristics. Based on this observation, it can be concluded that the isolated compound was quercetin (25, 28).

High Performance Thin Layer Chromatographic Method for Quantitative Determination of Quercetin in Tender Leaves of P. guajava

To quantify quercetin in P. guajava, the following parameters can be used:

Mobile phase: Toluene: Ethyl Acetate: Formic Acid (5:5:0.3 v/v/v) (25).

Development: Development chamber pre-saturated (20 min) with the mobile phase (25).

Source of radiation: Deuterium lamp 200-400 nm (25).

Detection: 254nm and Visible Light (25).

Preparation of the sample solution: The acetone extract of P. guajava displayed identical spots on CO-TLC when compared to the quercetin standard. Therefore, the acetone extract was utilized for the development of the HPTLC method. Approximately 10 mg of the guava extract was dissolved in methanol and then transferred to a standard flask. Methanol was added to reach a final volume, resulting in a concentration of 1000 µg/mL (29, 30).

Preparation of the standard: A 10 mg quantity of the quercetin standard was dissolved in methanol. This solution was subsequently transferred to a standard flask and diluted to obtain a concentration of 100 µg/mL.

Method specifications: Silica gel 60F 254 precoated plates measuring 10x10 cm were used with a solvent system consisting of toluene: acetone: formic acid (38:10:5 v/v/v). Using a Linomat 5 spotter, 10 and 20 µL of the samples from a 1000 µg/mL sample solution were applied as distinct tracks on the precoated TLC plates. The development process was conducted in ascending mode, progressing up to a distance of 8 cm. The resulting plates were dried, and a TLC scanner 3 was employed to scan the plate under a 364 nm laser. On the HPTLC chromatogram, the standard quercetin exhibited a single peak. A quercetin calibration curve was created by plotting the concentration of quercetin against the average peak area over the range of 200-1000 µg/band. The correlation coefficient was determined to be 0.98470 (26, 29).

Validation and Vitamin C Testing in Crystal Guava (P. guajava L.) With Variations of Origin with the HPLC Method (High Performance Liquid Chromatography)

Ingredients: Crystal guava (P. guajava) varieties, standard vitamin C with 99% purity (E-Merck), HPLC-grade aquabidest (Ika Farma), HPLC-grade methanol (E-Merck), KMnO4 pro analysis (E-Merck), FeCl3 pro analysis (E-Merck), AgNO3 pro analysis (E-Merck) (31, 32).

Equipment: Glassware, HPLC set (LC-20AT with SPD-20A SHIMADZU detector), analytical scale (Ohaus of PA214 type), centrifuge (Eickmeyer), sonicator (Elmasonic), juicer (Sapporo), micropipette (Socorex), blue tips, 1 mL syringe, millipore PVDF 0.22 µm filter (Sartoriusstedim), filter paper no. 1 and no. 42 (Whatman) (31, 32).

Slurry preparation: Crystal guava was cleaned and drained with running water. The peel, pulp, and seeds were separated, and the fruit's flesh was smoothed with a juicer.

Sample preparation: For the study, each region's crystal guava was cut into five pieces. After cleaning, the fruit's flesh was processed into a slurry with a juicer. Once the fruit has been processed, 10 grams of the sample was carefully weighed and it was placed in a 10 mL flask with the appropriate amount of aquabidest. It was centrifuged for 5 minutes at a speed of 5000 rpm. After centrifugation, the supernatant was filtered through a filter paper (Whatman no. 42) (31).

Determination of vitamin C: An extract of up to 100

µL of clear samples was taken into a 10 mL flask, and the appropriate amount of aquabidest was added. The samples were filtered with a 0.22 µm millipore paper. Then, 20 mL of the samples was injected into the HPLC system (31, 33).

Data analysis: The Area Under Curve (AUC) values of vitamin C in the samples was entered to calculate the levels of vitamin C with the regression line equation y = bx + a, where x is the standard concentration used to make the standard curve and y is the absorbance of the standard solution of vitamin C. This analysis was performed for each sample of guava crystals from Margajaya (Bogor), Junrejo (Malang), and Nglipar (Gu) (31).

HPLC Method Validation

System Suitability Test

Based on various metrics, the coefficient of variation (CV) for the retention time was 1.94 ± 2%, and the CV for the area under the curve (AUC) was 1.523 ± 5%. The average values for N and the tailing factor are 5.7% and 15%, respectively. These results indicate the strong system compatibility of the HPLC method (31, 34).

Linearity

The level of linearity between vitamin C levels and the AUC area was determined by the correlation coefficient or R-value. The calculated R-value for the linear regression equation y = 106053.1x + 431039 was 0.9911, indicating a strong linear relationship. Similarly, the linearity between the analyte concentration and the peak area was evaluated with the correlation coefficient or R-value, yielding a value of 0.9911. However, according to the AOAC, the suggested threshold for the correlation coefficient was less than 0.99. Despite this, the linear regression equation can still be employed to determine the amounts of vitamin C in the samples, as it satisfies the linearity conditions and demonstrates the effectiveness of the HPLC method for this test (31, 34).

Precision

CV values of 1.37%, 2.83%, and 0.93% were observed at concentrations of 1 mg/mL, 5 mg/mL, and 9 mg/mL, respectively. Since the CV result was below 5%, it confirms the accuracy of the procedure. These findings demonstrate that this technique meets the precision criteria (31).

Quantitative Analysis

This approach was chosen for its excellent selectivity, sensitivity, and rapid processing. The linear regression equation resulted in a high R-value of 0.9911, with the equation y = 106053.1x + 431039. Based on data analysis, the crystal guavas exhibited vitamin C contents of 0.8608 mg/mL, 0.6746 mg/mL, and 0.4139 mg/mL in South Mountain, Malang, and Bogor, respectively (31).

Pharmaceutics

A series of formulations have been developed from P. guajava. Mucoadhesive Herbal Buccal Patch of *P. guajava* L was used to treat mouth ulcers (35). Guava pulp methanolic extracts, increased sunscreen in the formulation by permitting the decrease of various pharmaceutical photoprotective substances and establishing a synergistic behaviour with other ingredients (36). Chewable guava extract tablets show anti-cariogenic action against *Streptococcus mutans* (37). Guava leaf extract was used on jelly sweets to prevent *S. mutans* from growing in sugar syrup with high moisture content (38). Herbal scrub of guava was used to treat wrinkles, redness, and acne on the skin (39). Guava leaf extracts hard candies are used to inhibit upper respiratory tract infection (40). Guava leaf extract herbal gel showed antibacterial and antifungal activity (41). A face wash gel was prepared with *P. guajava* seed extract which possesses antioxidant and antibacterial properties (42). The toothpaste containing an Alcoholic Extract of *P. guajava* Leaf was formulated (43). Guava extract capsule was formulated on heart function in dengue fever and dengue haemorrhagic fever patients (44).

Conclusion

P. guajava is one of the most “widely utilized” herbal medicine. Almost every part of the plant possesses a potential therapeutic effect on a wide range of diseases. The main purpose of this review was to elaborate the analytical method development on *P. guajava* species with reference to the identification, separation, and quantification of the active components that are responsible for its therapeutic activity. Through this literature, we recommend the development of a more robust analytical method that will be more cost-effective and time-saving.

Declarations

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Conflict of Interest
The authors declare no conflicting interest.

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